Claims

1. A method for assessing at least one quality parameter or at least one quantity parameter of a particle in a liquid material, said liquid material comprising particles having bound thereto or comprised therein at least one species of analytes in an amount of less than $1x10^6$ analyte detectable positions,

comprising:

mixing the liquid material with at least one reagent material, said reagent material at least comprising a first targeting species capable of selectively and directly binding to an analyte position of said species of analytes and a labelling agent, wherein the first targeting species and the labelling agent are directly or indirectly coupled to each other,

arranging a volume of a liquid material comprising at least part of the mixture of the liquid material and the reagent material in a sample compartment having a wall part defining an exposing area, the wall part allowing electromagnetic signals from the sample in the compartment to pass through the wall to the exterior,

exposing, onto an array of active detection elements, a representation of electromagnetic signals having passed through the wall part from the sample in the sample compartment, wherein the representation is subject to a linear enlargement, so that the ratio of the image of a linear dimension on the array of detection elements to the original linear dimension in the exposing domain is smaller than 20:1,

detecting the representation as intensities by individual active detection elements,

processing the intensities in order to identify representations of electromagnetic signals from the particles as distinct from representations of electromagnetic signals from background, and

obtaining the at least one quality parameter or at least one quantity parameter from the result of the processing.

2. The method according to claim 1, wherein the particle is selected from cells, cell walls, bacteria, plasmodia, virus, prions, or fragments or clusters thereof, and macromolecules and beads.

3. The method according to claim 2, whereby the particle is a bead, to which analytes are bound.

4. The method according to any of the preceding claims, whereby the analyte is selected from proteins, polypeptides, peptides, lipids, carbohydrates, lipoproteins, carbohydrate-conjugated proteins, membrane constituents, receptors, genes, DNA, RNA, or fragments or clusters thereof.

5. The method according to any of the claims 2-4, whereby the analyte is bound to a cell membrane or cell nucleus membrane, such as whereby the analyte is a cell receptor.

6. The method according to any of the claims 2-4, whereby the analyte is comprised in a cell.

7. The method according to claim 6, whereby the analyte is comprised inside an organelle.

8. The method according to claim 6, whereby the analyte is located on the surface of an organelle.

9. The method according to any of the preceding claims, whereby the particles have bound thereto or comprised therein at least one species of analytes in an amount of less than $5x10^5$ analyte detectable positions, such as less than $1x10^5$ analyte detectable positions, such as less than $5x10^4$ analyte detectable positions, such as less than $1x10^4$ analyte detectable positions, such as less than $5x10^3$ analyte detectable positions, such as less than $1x10^3$ analyte detectable positions, such as less than $5x10^2$ analyte detectable positions, such as less than $1x10^2$ analyte detectable positions, such as less than 10 analyte detectable positions, such as 1 or 2 analyte detectable position.

10. The method according to any of the preceding claims, whereby the particles have between 500 and 50,000 analyte detectable positions (average for population), more preferably between 1,000 and 10,000 analyte detectable positions (average for population) bound thereto or comprised therein.

11. The method according to any of the preceding claims, wherein the cells are selected from mammalian cells, insect cells, reptile cells, fish cells, yeast cells, and fungi cells.

12. The method according to any of the preceding claims, wherein the cells are selected from blood cells, sperm cells, and bone marrow cells.

13. The method according to any of the preceding claims, whereby the liquid material comprises at least two different species of particles.

14. The method according to claim 13, whereby only one of the species of particles has bound thereto or comprised therein the species of analyte.

15. The method according to any of the preceding claims, comprising binding at least two distinct targeting species to at least two distinct species of analyte and labelling the at least two distinct targeting species with two distinct labelling agents.

16. The method according to any of the preceding claims, whereby one species of analyte is selected from CD (Cluster of Differentiation) markers, such as CD3, CD4, CD8, CD16, CD19, CD22, CD34, CD45, CD61, and CD91, Epithelial Membrane Antigen (EMA), Estrogen receptor α (ERα), Cytokeratin Human, Cytokeratin 7, Cytokeratin 20, Ki-67/PI, Phosphatidylserine, BCL2 Oncoprotein, suPAR (soluble urokinase Plasminogen Activator Receptor), urokinase, a hormone bound to a receptor, a cell cycle related protein, a marker of apoptosis, and Green fluorescent protein (GFP).

17. The method according to any of the preceding claims, whereby one species of analyte is selected from a chromosomal DNA sequence, a mitochondrial DNA

sequence, a chloroplast DNA sequence, a mRNA sequence, a rRNA sequence, a nucleotide sequence comprising a single nucleotide polymorphism.

18. The method according to any of the preceding claims, whereby one species of analyte is a cell cycle related protein, e.g. cycline (such as cyclin D1), tumor suppresser protein (e.g. p53 protein), Epidermal Growth Factor protein (EGF protein), Transforming Growth Factor beta (TGF-beta1), Ki-67 protein.

19. The method according to any of the preceding claims, whereby the analyte ia a cell cycle related protein receptor such as Epidermal Growth Factor Receptor (EGFR), Cyclin Dependent Kinases (e.g. CDK4).

20. The method according to any of the preceding claims, whereby one species of analyte is a marker of apoptosis, e.g. membrane bound phosphatidylserines, phosphatidylserines targeted with Annexin V, BCL2 oncoprotein.

21. The method according to any of the preceding claims, whereby the at least one species of analyte is a medical marker of a disease.

22. The method according to claim 21, whereby the disease is selected from cancer, genetic diseases, heart diseases, infectious diseases, immune-related diseases.

23. The method according to claim 21, whereby the disease is selected from breast cancer, ovarian cancer, epithelial carcinoma, leukemia, sepsis.

24. The method according to claim 21, whereby the disease is an infectious disease and the particle is selected from vira such as HIV, Hepatitis virus, Herpes virus, Epstein-Barr virus, Human Papilloma Virus (HPV), chlamydia virus, cytomegalovirus (CMV), influenza virus.

25. The method according to claim 24, whereby the analyte is an antigen located in a cell infected by said virus.

26. The method according to claim 24, whereby the analyte is located in or on the virus, said antigen being selected from the group comprising a nucleotide sequence, a coat protein.

27. The method according to any of the preceding claims, whereby the reagent material comprises more than one first targeting species, each of said targeting species being directed to a different analyte.

28. The method according to claim 27, whereby the different targeting species are mixed with the liquid material simultaneously.

29. The method according to claim 27, whereby the different targeting species are mixed with the liquid material sequentially.

30. The method according to any of the preceding claims, whereby the targeting species is an antibody directed to the analyte species, such as an antibody selected from a monoclonal antibody, a polyclonal antibody and/or a chimeric or synthetic antibody.

31. The method according to any of the preceding claims, whereby the targeting species is a nucleotide probe complementary to a sequence of an analyte species.

32. The method according to claim 31, wherein the nucleotide probe comprises monomers selected from the group comprising DNA, RNA, PNA, LNA and modified analogues thereof.

33. The method according to any of the preceding claims, whereby the targeting species is an In situ hybridisation (ISH) probe, preferably a fluorescent in situ hybridisation probe (FISH).

34. The method according to any of the preceding claims, wherein the liquid material is selected from body fluids, such as blood, urine, saliva, bile, sperm, faeces, cerebro-spinal fluid, nasal secrete, tears, bone marrow, and milk, milk products,

waste water, process water drinking water, food, feed, and mixtures, dilutions, or extracts thereof.

35. The method according to any of claims 1-34, whereby the liquid material is prepared from a solid or semi-solid sample, which is pre-treated prior being arranged in the sample domain.

36. The method according to claim 35, whereby the solid or semi-solid material is a biopsy of a muscle, a brain, a kidney, a liver, a spleen.

37. The method according to any of the preceding claims, wherein the reagent material is selected from immunoreactive solutions, tissue fixative solutions.

38. The method according to any of the preceding claims, wherein the reagent material is selected from blocking agents (e.g. Bovine Serum Albumin), surfactant polymers or surfactant co-block polymers (e.g. Pluronic polymers), detergents, dextrans, dextransulphates, sugars, or polymerised products made thereof.

39. The method according to any of the preceding claims, wherein the reagent material comprises Tween 20, and/or formaldehyde, and/or ethanol, and/or methanol, and/or acetone, and/or a fixative acid such as picric acid or citric acid.

40. The method according to any of the preceding claims, wherein the pH of the mixture is from 4 to 10, such as from 4-5, from 5-6, from 6-7, from 7-8, from 8-9, from 9-10.

41. The method according to any of the preceding claims, wherein the concentration of NaCl is from 0.1 gram/L to 50 gram/L, such as from 0.1 g/L to 1 g/L, from 1 g/L to 2 g/L, from 2 g/L to 5 g/L, from 5 g/L to 10 g/L, from 10 g/L to 10 g/L, from 20 g/L to 30 g/L, from 30 g/L to 40 g/L, from 40 g/L to 50 g/L.

42. The method according to any of the preceding claims, wherein the temperature during hybridisation or specific binding is between 10 and 90°C, such a between 10 and 15°C, from 15 to 20°C, from 20 to 25°C, from 25 to 30°C, from 30 to

35°C, from 35 to 40°C, from 40 to 45°C, from 45 to 50°C, from 50 to 60°C, from 60 to 70°C, from 70 to 80°C, from 80 to 90°C.

43. The method according to any of the preceding claims, wherein the labelling agent is selected from fluorescently labelled antibodies (e.g. anti-CD3/FITC), antibodies labelled with reactive molecules (e.g. anti-CD3/biotin).

44. The method according to any of the preceding claims, wherein the labelling agent is selected from fluorescently labelled nucleotide probes (e.g. C-MYC cDNA-FITC), nucleotide probes labelled with reactive molecules.

45. The method according to any of the preceding claims, wherein the reagent material further comprises lysing agents and tissue fixative agents.

46. The method according to any of the preceding claims, wherein the reagent material further comprises fluorescence quenching agents, light absorbing agents, fluorescence amplification agents. (e.g. fluorescyl-tyramine, Cy3-tyramine)

47. The method according to any of the preceding claims, wherein the mixture of the liquid material and the reagent material is diluted before being arranged in the sample compartment.

48. The method according to claim 47, wherein the mixture is diluted to at least 1:2, such as at least 1:3, such as at least 1:4, such as at least 1:5, such as at least 1:10, such as at least 1:20, such as at least 1:100.

49. The method according to any of the preceding claims, whereby the labelling agent is selected from agents giving rise to one or several of the following phenomena: attenuation of electromagnetic radiation, photoluminescence when illuminated with electromagnetic radiation, scatter of electromagnetic radiation, raman scatter.

50. The method according to claim 49, whereby the labelling agent is selected from fluorescein (FITC), phycoerythrin, (RPE or PE), cyanine dyes (Cy dyes), Cy3,

Cy5, Cy5.5, allophycocyanines (APC), indotrimethinecyanines, indopentamethinecyanines, acridine orange, thiazole orange, DAPI, propidium iodide (PI), ethidium iodide, 7-aminoactinomycin D, Per CP or chemically coupled combinations thereof.

51. The method according to claim 50, wherein the chemically coupled combination is selected from RPE-Cy5, and PerCP-Cy5.5.

52. The method according to any of the preceding claims, whereby the sample domain is three-dimensional.

53. The method according to any of the preceding claims, whereby the sample domain is a flow through chamber.

54. The method according to any of the preceding claims, whereby the sample domain is part of a disposable cassette.

55. The method according to any of the preceding claims, whereby the recording of image comprises the use of a confocal scanner.

56. The method according to any of the preceding claims, whereby the image is recorded using an array of detection devices.

57. The method according to any of the preceding claims, wherein the image is recorded using a one-dimensional array of detection devices.

58. The method according to any of the preceding claims 1 to 57, wherein the image is recorded using a two-dimensional array of detection devices.

59. The method according to any of the preceding claims, wherein the image is recorded using a CCD, a CMOS, a video camera or a photon counting camera.

60. The method according to any of the preceding claims, whereby the image is recorded without enlargement.

61. The method according to any of the preceding claims, whereby the enlargement ratio is below 10, more preferably below 5, such as 4, more preferably below 4 such as 2, more preferably below 2, such as 1.

62. The method according to any of the preceding claims, whereby the enlargement ratio is below 1, preferably below 0.9, such as 0.8, more preferably below 0.8 such as 0.6, more preferably below 0.6 such as 0.5.

63. The method according to any of the preceding claims whereby the image is recorded in one exposure.

64. The method according to any of the claims 1-62 whereby the image is recorded in two, three or more exposures.

65. The method according to claim 64, wherein the assessment of the number of particles is obtained on the basis of more than one image, preferably two images, more preferably more than two images, more preferably more than four images.

66. The method according to claim 64, where information about the changes in the image in course of time is used in the assessment of the number of particles.

67. The method according to any of the preceding claims, whereby the recorded image is processed using data processing means.

68. The method according to claim 67, whereby the data processing means distinguish partially overlapping areas of labelling agent.

69. The method according to any of the preceding claims, whereby a distinction between at least two spectral properties of a labelling agent is used to obtain the at least one quality parameter or at least one quantity parameter of the particles.

70. The method according to any of the preceding claims, whereby the recording of an image further comprises exposing a first surface of the sample directly with excitation light from a first light means having at least a first light source, by use

of focusing means detecting a fluorescence signal from the first surface of the sample onto a first detection means comprising at least a first detector.

71. The method according to claim 70, wherein at least the first light means is located in a first light plane parallel to the sample plane, said first light plane being between the sample plane and the first detection means.

72. The method according to claim 70, wherein the excitation light is a light source selected from the group of, light emitting diode (LED), gas laser, solid state laser, laser diode, gas lamp, halogen lamp, xenon lamp.

73. The method according to any of the preceding claims 70-72, wherein an excitation light filter is inserted in the excitation light path from at least one light source.

74. The method according to claim 70, wherein the excitation light is arranged as light sources on a supporting material.

75. The method according to any of the preceding claims 70-74, wherein substantially identical filters are used for all the light sources.

76. The method according to any of claims 70-75, wherein a first light source is filtered through a first filter, and a second light source is filtered through a second filter, the first filter and the second filter being different.

77. The method according to any of the preceding claims 70-76, further comprising exposing a second surface of the sample directly with excitation light from a second light means having at least one light source.

78. The method according to claim 77, wherein the second excitation light means is located in a second light plane said plane being parallel with the sample plane and located on the other side of the sample plane than the first light plane allowing the sample to be exposed on two opposite surfaces.

79. The method according to claim 77 or 78, wherein a filter inserted in the light path from the second light means is different from a filter inserted in the light path of the first light means.

80. The method according to any of claims 70-79, wherein a second detection means is arranged so that the sample compartment is positioned between the first detection means and the second detection means.

81. The method according to claim 80, wherein the first detection means is identical with the second detection means.

82. The method according to any of the preceding claims 70-81, wherein an emission light filter is inserted in the emission light path to at least the first detector.

83. The method according to any of the preceding claims 70-82, wherein a collimating lens is arranged in the emission light path.

84. The method according to any of the preceding claims 70-83, wherein the angle between the excitation main light and the detection-sample axis is in a range between 0° and 90°, to the optical axis of the detection system, more preferably between 0° and 60°, such as between 10° and 45°.

85. The method according to claim 84, wherein at least the first light means is located in a first light plane parallel to the sample plane, said first light plane being positioned at a distance from the sample plane behind the detector.

86. The method according to claim 85, wherein the detector is positioned in a housing having an opening allowing the emitted signals to reach the detector(s).